Radiation Sensitivity and Postirradiation Growth of Foodborne Pathogens on a Ready-to-Eat Frankfurter on a Roll Product in the Presence of Modified Atmosphere and Antimicrobials[†]

CHRISTOPHER H. SOMMERS* AND GLENN BOYD

Food Safety Intervention Technologies Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

ABSTRACT

Intervention technologies including ionizing radiation, antimicrobials, and modified atmospheres (MA) can be used to inhibit the growth of or inactivate foodborne pathogens on complex ready-to-eat foods such as sandwiches. However, the effect of these technologies when used in combination (the hurdle concept) on the survival of foodborne pathogens is unknown. The ability of ionizing radiation to inactivate *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus* inoculated onto a frankfurter on a roll product containing the antimicrobials sodium diacetate and potassium lactate in the presence of an MA (100% N_2, 50% N_2 plus 50% CO_2, or 100% CO_2) was investigated. The radiation resistances (D_{10}-values) of the foodborne pathogens were 0.43 to 0.47 kGy for *E. coli* O157:H7, 0.61 to 0.71 kGy for *Salmonella*, 0.53 to 0.57 for *L. monocytogenes*, and 0.56 to 0.60 for *S. aureus*. The MA had no effect on the radiation resistance of the pathogens. During a 2-week storage period under mild temperature abuse (10°C), none of the pathogens were able to proliferate on the frankfurter on a roll product, regardless of the MA used. However, application of sublethal doses of ionizing radiation resulted in increased mortality of the gram-positive pathogens *L. monocytogenes* and *S. aureus* during the storage period regardless of the MA. Although the pathogens were unable to proliferate on the frankfurter on a roll product during the storage period, application of a postpackaging intervention step was needed to actually inactivate the foodborne pathogens. Ionizing radiation used in combination with sodium diacetate and potassium lactate resulted in additional mortality of *L. monocytogenes* and *S. aureus*, independent of the MA, during the 2-week storage period.

Sales of sandwich products from vending machines are a multi-billion-dollar business in the United States *(2, 4)*. Refrigerated ready-to-eat (RTE) sandwich products available for purchase from either retail stores or vending machines have relatively short shelf lives, ranging from 24 to 96 h. Because RTE sandwiches are multiingredient products that are assembled after production or cooking of their individual components, they are particularly vulnerable to postprocess contamination with foodborne pathogens *(27)*. This postprocess contamination is especially important because sandwich products may receive little or no heating prior to consumption by consumers. Examination of food product recalls and foodborne illness outbreaks listed on Web sites maintained by the U.S. Food and Drug Administration (FDA), the U.S. Food Safety Inspection Service, and the Centers for Disease Control and Prevention (CDC) indicates that a wide variety of pathogenic bacteria and viruses are associated with those events *(3, 25, 26)*. Therefore, prepackaged sandwich products could benefit from exposure to a postpackage intervention, as part of a hazard analysis and critical control point plan, to inactivate or pre-

vent the growth of pathogenic microorganisms that cause foodborne illness, especially in the large at-risk portion of the population *(8, 19)*.

Ionizing radiation (IR) has been shown to be a safe and effective method for inactivating foodborne pathogens on a variety of food products, including RTE sandwiches and ready-meals, and a petition to allow irradiation of RTE foods is currently being evaluated by the FDA *(5, 7, 11, 13, 15, 20, 21)*. Generally recognized as safe (GRAS) food additives such as sodium diacetate (SA) and potassium lactate (PL) or a mixture of the two can be included in the formulation of food products to inhibit the growth of foodborne pathogens, increase pathogen sensitivity to IR, and prevent the growth of radiation-damaged pathogens during refrigerated storage under mild temperature abuse conditions *(22, 23)*. Modified atmosphere (MA) environments, especially those with high concentrations of CO_2, have been reported to increase the radiation sensitivity of foodborne pathogens and delay the growth of foodborne pathogens during refrigerated storage *(1, 9, 16)*. However, very little information exists on the use of IR, MAs, and the GRAS food additives SA and PL to control pathogenic bacteria inoculated onto complex RTE foods.

The purpose of this study was to investigate the feasibility using IR to inactivate the pathogenic bacteria *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella*, and *Escherichia coli* O157:H7 inoculated onto a precooked

* Author for correspondence. Tel: 215-836-3754; Fax: 215-233-6445; E-mail: csommers@errc.ars.usda.gov.
† Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

TABLE 1. *Radiation D_{10}-values of foodborne pathogens inoculated onto frankfurter on a roll products under modified atmospheres[a]*

Pathogen	100% N_2		50% N_2 + 50% CO_2		100% CO_2	
	D_{10} (kGy)	R^2	D_{10} (kGy)	R^2	D_{10} (kGy)	R^2
E. coli O157:H7	0.47 (±0.01)	0.98	0.43 (±0.01)	0.99	0.43 (±0.01)	0.97
Salmonella	0.70 (±0.02)	0.98	0.71 (±0.02)	0.98	0.61 (±0.03)	0.92
L. monocytogenes	0.62 (±0.02)	0.96	0.53 (±0.02)	0.95	0.57 (±0.02)	0.97
S. aureus	0.60 (±0.02)	0.97	0.56 (±0.03)	0.92	0.58 (±0.01)	0.98

[a] D_{10}-values are mean (±SD) (n = 3, α = 0.05).

frankfurter (hot dog) on a roll (FOAR) product in the presence of three MAs: 100% N_2, 100% CO_2, and 50% N_2 plus 50% CO_2. Commercially available frankfurters that contained SA and PL to delay the growth of pathogenic bacteria were used in the study, and the products were wrapped in oxygen-permeable packaging. The ability of the foodborne pathogens to proliferate on the FOAR following application of sublethal doses of IR under conditions of mild temperature abuse (10°C) for 2 weeks after irradiation also was investigated.

MATERIALS AND METHODS

Bacterial strains. *L. monocytogenes* strains H7762, H7962, and H7969 were obtained from the CDC (Atlanta, Ga.). *S. aureus* strains 25923, 13565, and 14458, *Salmonella* Enteritidis 13076, *Salmonella* Typhimurium, *Salmonella* Newport 6962, and *E. coli* O157:H7 35150 were obtained from the American Type Culture Collection (Manassas, Va.). *E. coli* O157:H7 ENT C9490 was obtained from the CDC, and *E. coli* O157:H7 93-437 was from the Oregon Public Health Laboratory (Portland, Oreg.). Identity of the isolates was confirmed by gram stain, followed by analysis with gram-positive or -negative identification cards using the Vitek Automicrobic System (bioMérieux Vitek, Inc., Hazelwood, Mo.). The bacterial strains were cultured on tryptic soy agar (TSA; BBL, Becton Dickinson, Sparks, Md.) at 37°C and were maintained at 0 to 4°C until use.

RTE foods. RTE foods were purchased from local retail outlets. Frankfurter rolls consisted of bleached enriched wheat flour, water, high-fructose corn syrup, yeast, soybean oil, wheat gluten, salt, calcium propionate, mono- and diglycerides, sodium stearol lactylate, grain vinegar, calcium sulfate, calcium peroxide, fumaric acid, monocalcium phosphate, azodicarbonamide, and soy lecithin. Frankfurters consisted of beef, pork, water, salt, flavoring, paprika, sodium phosphate, sodium diacetate (0.07%), PL (1.13%), sodium erythorbate, and sodium nitrate. Frankfurters were cooked to an internal temperature of 70°C before being placed on a frankfurter roll. Examination of frankfurters sold in vending machines indicated the use of reheated (cooked) frankfurters in the actual commercial products.

Preparation of inoculum. The procedure of Sommers and Boyd *(20, 21)* was used for inoculation, irradiation, and enumeration of bacteria. Each bacterial strain was cultured independently in 100 ml of tryptic soy broth (BBL, Becton Dickinson) in baffled 500-ml Erlenmeyer culture flasks at 37°C (150 rpm) for 18 h. The cells (30 ml of culture per isolate) were then pelleted by centrifugation and resuspended as a same-species mixture in 9 ml of Butterfield's phosphate buffer (BPB; Applied Research Institute, Newtown, Conn.).

Inoculation. The assembled products were cut into equal quarters (approximately 25 g) and then inoculated with 0.1 ml (10^9 CFU) of each bacterial species mixture, which was distributed onto the components (bread and frankfurter) of the FOAR product with a premoistened sterile swab. Noninoculated products were also assembled to measure background microflora. The inoculated quarters were then placed in FDA-approved polyethylene radiation bags (175 by 304 mm; wall thickness: 65 μm; oxygen transmission: PO_2 5,000 $cm^3/m^2/24$ h/ATM at 23°C) (Seward Ltd., London, UK), and after a gas flush the bags sealed with an A300 packager (Multi-Vac, Kansas City, Mo.) under MA conditions with premixed gases (Scott Gases, Perkiomenville, Pa.).

The same inoculation procedure was used for the storage studies except that microorganism concentrations were adjusted such that ≤10^3 CFU/g would be obtained following application of IR. Radiation doses were selected based on the D_{10}-values to provide a 2.0- to 2.5-log reduction of pathogen: 1.5 kGy for *Salmonella*, *L. monocytogenes*, and *S. aureus* and 0.75 kGy for *E. coli* O157:H7. After irradiation, the samples were stored at 10°C for 2 weeks to simulate mild temperature abuse.

Gamma radiation. A self-contained ^{137}Cs radiation source (Lockheed Georgia Company, Marietta, Ga.) was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The cylindrical sample chamber (22.9 by 63.5 cm) was central to the array when placed in the operating position. The dose rate was 0.092 kGy/min. The temperature during irradiation was maintained at 4.0 (±1.0)°C by the gas phase of a liquid nitrogen source that was introduced directly into the top of the sample chamber. To ensure that a uniform radiation dose was delivered, sample bags were placed centrally and vertically within the cylindrical chamber. Dose uniformity ratios were less than 1.1:1.0 for the 25-g sample size and geometry. The temperature was monitored with two thermocouples placed on the side of the sample bags. Four incremental radiation doses, depending on previously determined radiation D_{10}-values for the individual pathogens, up to a maximum of 2.5 kGy were used. The dose delivered was verified by use of 5-mm alanine pellet dosimeters that were attached to the side of the sample bags. Dosimeter data were interpreted with a EMS 104 EPR analyzer (Bruker BioSciences, Billerica, Mass.). Recorded doses were typically ±5% of the target doses. Radiation doses used ranged from 0 to 2.5 kGy depending on the bacterium.

Enumeration of bacteria. One day (24 h) after irradiation, the samples were assayed for surviving bacteria by standard pour plate procedures. A 25-g inoculated sample was stomached for 90 s with 100 ml of sterile BPB in a no. 400 stomacher bag. The samples were then serially diluted in BPB, making 10-fold dilutions, and 1 ml of diluted sample was pour plated onto Palcam agar (for *L. monocytogenes*), Baird-Parker agar (for *S. aureus*), Hektoen agar (for *Salmonella*), or MacConkey agar (for *E. coli*

O157:H7) (BBL, Becton Dickinson). Previous research *(20, 21)* has indicated the selective media used in this study do not prevent the recovery and growth of their respective injured bacteria. Two 1-ml aliquots were plated per dilution. The plates were then incubated for 48 h at 37°C. Noninoculated nonirradiated samples were used to determine aerobic plate counts by pour plating on TSA, which indicated that background microflora concentrations were less than 2 log CFU/g.

D_{10}-values. The average concentration of an irradiated sample (N) was divided by the average concentration of the untreated control (N_0) to produce a survivor ratio (N/N_0). The D_{10}-value is defined as the radiation dose required to achieve a 90% reduction in viable microorganisms. D_{10}-values were determined by calculating the reciprocal of the slope of the log (N/N_0) ratios versus radiation dose *(6)*.

Head space gas analysis. Head space gas analysis was performed with a Gow-Mac 580 series gas chromatograph (Alltech Associates, Inc., Deerfield, Ill.). Performance of the gas chromatograph was verified with air and Scotty I Standard Mix 237 (Scott Gases). Each sample bag was tested for maintenance of the MA before microbiological testing. The gas:sample volume ratio was approximately 3:1.

Statistical analysis. Each experiment was conducted independently three times. D_{10}-values, descriptive statistics, and analysis of variance (ANOVA) were generated with Microsoft Excel Office 2000 (Microsoft Corp., Redmond, Wash.).

RESULTS AND DISCUSSION

The radiation resistance of the individual foodborne pathogens inoculated onto the FOAR product was not affected by the presence of the MA as determined by ANOVA ($n = 3$, $\alpha = 0.05$) (Table 1). The D_{10}-values were 0.41 to 0.43 kGy for *E. coli* O157:H7, 0.61 to 0.71 kGy for *Salmonella*, 0.53 to 0.62 kGy for *L. monocytogenes*, and 0.56 to 0.60 kGy for *S. aureus*. The lack of significant differences in D_{10}-values was not due to the loss of the MA in the gas-permeable polyethylene packaging before irradiation. The samples were irradiated 1 to 2 h after application of the MA, and the gas composition did not return to the normal atmospheric composition until 48 to 72 h postirradiation (Fig. 1). There was no advantage to using an MA in combination with gas-permeable packaging for pathogen reduction purposes in this study. The order of radiation resistances (D_{10}-values) of the foodborne pathogens, from the most resistant to the least resistant, was the same as that identified by Sommers and Boyd *(21)* and Thayer et al. *(24)* when the pathogens were inoculated into RTE sandwiches or raw meat products: *Salmonella* > *L. monocytogenes* $\geq$ *S. aureus* > *E. coli* O157:H7. A low radiation dose of 1.5 kGy could inactivate approximately 2 log CFU of *Salmonella*, 2.5 log CFU of *L. monocytogenes*, and *S. aureus*, and 3 log CFU of *E. coli* O157:H7 inoculated onto an FOAR product under an MA.

IR or lack of IR did not affect the proliferation of gram-negative *E. coli* O157:H7 and *Salmonella* during 2 weeks storage at 10°C, which is expected for refrigeration conditions or very mild temperature abuse *(12)* (Fig. 2). However, nonirradiated product contaminated with *E. coli* O157:H7 or *Salmonella* would still pose a hazard to con-
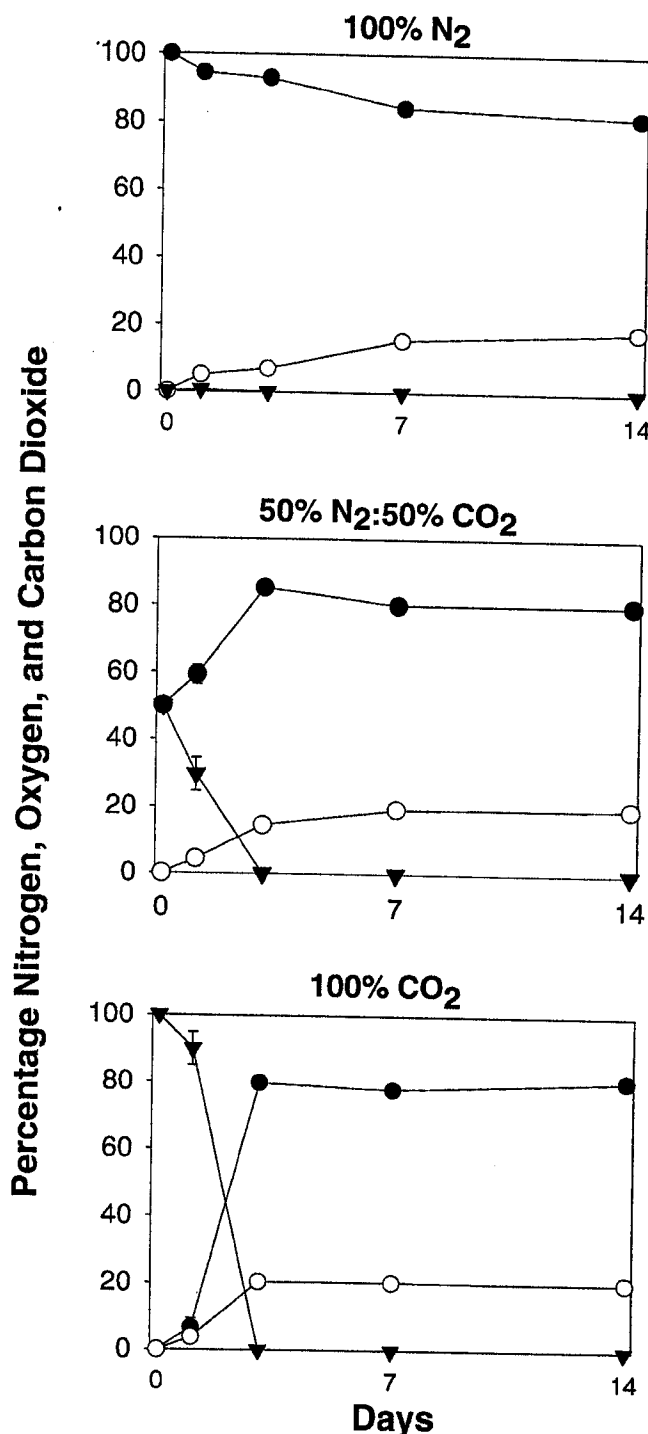

FIGURE 1. *Gas composition of packages containing a frankfurter on a roll product treated with modified atmospheres of 100% N_2, 50% N_2 plus 50% CO_2, or 100% CO_2 for 2 weeks after exposure to ionizing radiation. Three samples were evaluated at each time point. ●, nitrogen; ○, oxygen; ▼, carbon dioxide. Standard errors of the mean are shown by error bars.*

sumers because these pathogens, although not proliferating, were not inactivated. *E. coli* O157:H7 increased in the samples packaged in 100% CO_2; however, the increase was not significant because of the large variation in pathogen concentrations between experiments. Like nonirradiated *E. coli* O157:H7 and *Salmonella*, nonirradiated *L. monocytogenes*
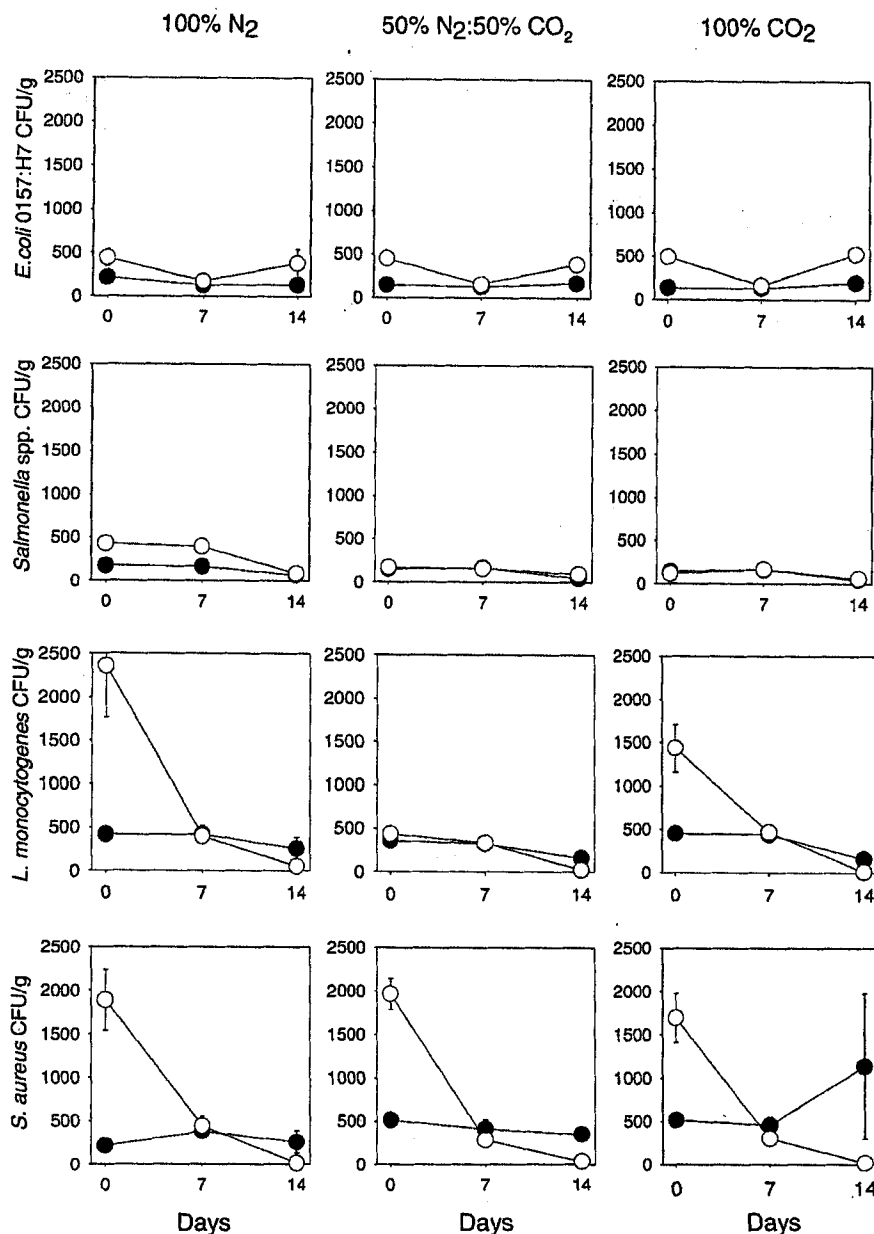
FIGURE 2. *Proliferation of irradiated (○) and nonirradiated (●) E.* coli *O157:H7, Salmonella,* L. monocytogenes, *and S. aureus inoculated onto frankfurter on a roll product during a 2-week period in the presence of a modified atmosphere. Each experiment was conducted independently three times. Standard errors of the mean are shown by error bars.*

and *S. aureus* did not proliferate during storage at 10°C but also were not inactivated. On the irradiated FOAR samples, however, the gram-positive pathogens *L. monocytogenes* and *S. aureus* decreased after 2 weeks of storage to levels that were not detectable with the pour-plate procedures used in this study (Fig. 2). Irradiation negatively impacted the survival of *L. monocytogenes* and *S. aureus* during storage under mild temperature abuse conditions.

Low doses of IR (≤5 kGy) have been used to inactivate pathogens on sandwiches consisting of meat, vegetable, cheese, and bread products (5, 7, 10, 11, 20, 21). SA and PL can inhibit the growth of foodborne pathogens in meat and RTE products during refrigerated storage (14, 17, 18). IR when used in combination with SA and PL increases the radiation sensitivity of foodborne pathogens and inhibits their proliferation during refrigerated storage under conditions of mild temperature abuse (22, 23). Previous research has also demonstrated the extreme radiosensitivity

of these four pathogens inoculated onto bread products (1). Although results of other studies have indicated that an MA could increase the effectiveness of IR (1, 9, 16), no such effect was observed in this study, in which gas-permeable packaging was used to prevent formation of an anaerobic environment favorable for germination and proliferation of *Clostridium botulinum*. Use of low-dose IR in combination with antimicrobials such as SA and PL, as demonstrated by previous research, can inactivate common foodborne pathogens on RTE food products, such as FOAR, commonly sold in convenience stores and vending machines even under mild temperature abuse conditions and can provide microbiologically safer convenience type foods for consumers.

REFERENCES

1. Al-Hadad, K. S. H., R. A. S. Al-Qassemi, and R. K. Robinson. 2005. The use of gaseous ozone and gas packaging to control populations of *Salmonella infantis* and *Pseudomonas aeruginosa* on the skin of chicken portions. *Food Control* 16:405–410.

2. Anonymous. 2001. Hot stuff. Food service sample, from Rich Products, spring 2001. TCP Publishing, Buffalo, N.Y.

3. Anonymous. 2002. Foodborne outbreak response and surveillance unit. U.S. foodborne disease outbreaks, 1990 to 2002. Available at: http://www.cdc.gov/foodborneoutbreaks/us_outb.htm. Accessed October 2005.

4. Anonymous. 2003. It's what's for dinner. *Prepared Foods* 172(4): 24.

5. Clardy, S., D. M. Foley, F. Caporaso, M. L. Calicchia, and A. Prakash. 2002. Effect of low-dose gamma irradiation on *Listeria monocytogenes* in frozen, artificially contaminated sandwiches. *J. Food Prot.* 65:1740–1744.

6. Diehl, J. F. 1995. Safety of irradiated food, 2nd ed., p. 93–98. Marcel Dekker, New York.

7. Foley, D. M., E. Reher, F. Caparaso, S. Trimboli, Z. Musherraf, and A. Prakash. 2001. Elimination of *Listeria monocytogenes* and changes in physical and sensory qualities of a prepared meal following gamma irradiation. *Food Microbiol.* 18:193–204.

8. Gerba, C. P., J. B. Rose, and C. N. Haas. 1996. Sensitive populations: who is at risk. *Int. J. Food Microbiol.* 30:113–123.

9. Grant, I. F., and M. Patterson. 1991. Effect of irradiation and modified atmosphere packaging on the microbial safety of minced pork stored under temperature abuse conditions. *Int. J. Food Sci. Technol.* 26:521–533.

10. Grecz, N., R. Bronnon, R. Jaw, R. Al-Harithy, and E. W. Hahn. 1985. Gamma processing of Arabic bread for immune system–compromised cancer patients. *Appl. Environ. Microbiol.* 50:1531–1534.

11. International Atomic Energy Agency. 2003. Radiation processing for safe, shelf-stable and ready-to-eat food. Proceedings of a final research co-ordination meeting held in Montreal, Canada, 10 to 14 July 2000. International Atomic Energy Agency, Vienna.

12. Jol, S., A. Kassianenko, K. Wszol, and J. Oggel. 2006. Process control: issues in time and temperature abuse of refrigerated foods. *Food Saf. Mag.* December 2005/January 2006, part 1–2. Available at: http://www.foodsafetymagazine.com/issues/0512/featcol.htm. Accessed April 2006.

13. Lamb, J. L., J. M. Gogley, M. J. Thompson, J. R. Solis, and S. Sumit. 2002. Effect of low dose gamma irradiation on *Staphylococcus aureus* and product packaging in ready-to-eat ham and cheese sandwiches. *J. Food Prot.* 65:1800–1805.

14. Mbandi, E., and L. A. Shelef. 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella* Enteritidis in meat by combinations of sodium lactate and diacetate. *J. Food Prot.* 64:640–644.

15. McAteer, N. J., I. R. Grant, M. F. Patterson, M. H. Stevenson, and S. T. Weatherup. 1995. Effect of irradiation and chilled storage on the microbiological and sensory quality of a ready-meal. *Int. J. Food Sci. Technol.* 30:757–771.

16. Metaragas, M., E. H. Drasinas, and J. Metaxaopoulos. 2003. Antagonistic activity of lactic acid bacteria against *Listeria monocytogenes* in sliced cooked cured pork shoulder under vacuum or modified atmosphere. *Food Microbiol.* 20:259–265.

17. Porto, A., A. Bernadette, B. Franco, E. Sant'Anna, J. Call, A. Piva, and J. Luchansky. 2002. Viability of a four strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, commercially prepared with and without 2.0 or 3.0% added potassium lactate during extended storage at 4 and 10°C. *J. Food Prot.* 65:308–315.

18. Shelef, L. A., and L. Addala. 1994. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. *J. Food Saf.* 14:103–115.

19. Smith, J. L. 1998. Foodborne illness in the elderly. *J. Food Prot.* 61:1229–1239.

20. Sommers, C. H., and G. Boyd. 2005. Elimination of *Listeria monocytogenes* from turkey and cheese tortilla wraps using ionizing radiation. *J. Food Prot.* 68:164–167.

21. Sommers, C. H., and G. Boyd. 2006. Variations in the radiation sensitivity of foodborne pathogens associated with complex ready-to-eat foods. *J. Radiat. Phys. Chem.* 75:773–778.

22. Sommers, C. H., and X. Fan. 2003. Irradiation (gamma) of fine emulsion sausage that contained sodium diacetate. *J. Food Prot.* 66: 819–824.

23. Sommers, C. H., X. Fan, B. A. Niemira, and K. Sokorai. 2003. Radiation (gamma) resistance and postirradiation growth of *Listeria monocytogenes* suspended in beef bologna that contained sodium diacetate and potassium lactate. *J. Food Prot.* 66:2051–2056.

24. Thayer, D. W., G. Boyd, J. Fox, L. Lakritz, and J. W. Hampson. 1995. Variations in radiation sensitivity of food-borne pathogens associated with the suspending meat. *J. Food Sci.* 60:63–67.

25. U.S. Food and Drug Administration. 2004. U.S. FDA enforcement report index, 1995 to 2004. Available at: http://www.fda.gov/opacom/Enforce.html. Accessed October 2004.

26. U.S. Food and Drug Administration, Food Safety and Inspection Service. 2004. FSIS recalls database. Available at: http://www.fsis.usda.gov/Fsis_Recalls/index.asp. Accessed October 2004.

27. Wilson, I. G. 1996. Occurrence of *Listeria monocytogenes* in prepacked retail sandwiches. *Epidemiol. Infect.* 117:89–93.